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Effect of Cypermethrin 25% EC, an Endocrine Disrupting Chemical and pH on Reproductive Health of *Puntius javanicus*

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Abstract: In the present study an attempt has been made to investigate the combined effect of sub-lethal dose of Cypermethrin 25% EC and pH on histoarchitecture of gonads and GnRH hormone of *Puntius javanicus*. 96h LC₅₀ value was determined by method of Karber and it was found to be 3.65 µg/L for *Puntius javanicus*. Adult fish were exposed to sub-lethal concentration (0.365 µg/L) of Cypermethrin 25% EC in different pH of water, i.e. pH 5, pH 6, pH 7, pH 8.5 and pH 9.5 for 15 days. Significant histopathological alterations were found in testis and ovary of the fish subjected to sub-lethal concentration of cypermethrin 25% EC. Significant decrease ($p < 0.001$) of GnRH concentration was recorded in pesticide exposed fishes. Significantly more histopathological alterations of gonads and decrease of GnRH concentration were recorded when fish were exposed to sub-lethal concentration of cypermethrin 25% EC in pH 5, pH 6 and pH 9.5, compared to fish exposed to sub-lethal concentration of cypermethrin 25% EC in pH 7 water. A significant increase of GnRH concentration ($p < 0.05$) was documented in fishes exposed to sub-lethal concentration of cypermethrin in pH 8.5, compared to fishes exposed to sub-lethal concentration of cypermethrin in pH 7 and the concentration is found to be almost equal to the control group (pH 7, without toxicant). At this medium histoarchitecture of testis and ovary retained almost normal histology with least alterations.

Keywords: Cypermethrin, Histopathology, Histoarchitecture, GnRH, Sub-lethal concentration, *Puntius javanicus*

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Introduction

Use of pesticides has been increased many fold to protect the crop from pests and to increase the production of crop (Aktar *et al.*,

2009; Chandola *et al.*, 2011; Gupta *et al.*, 2012; Srivastav *et al.*, 2020; Tudi *et al.*, 2021). The natural water reservoirs are getting polluted

all over the world due to injudicious use of pesticides (Gilliom *et al.*, 2007; Ngidlo, 2013). Pesticides affect overall ecosystem, especially the aquatic ecosystem, leading to undesirable mortality of aquatic flora and fauna, mainly fishes (Kumari, 2005; Gupta *et al.*, 2013; Srivastav *et al.*, 2020). Synthetic pyrethroids have been introduced over the past few decades for agricultural and domestic use. Pyrethroid pesticides adversely affect fish health (Sanchez- Fortun *et al.*, 2005). The biochemical changes in different organs/tissues of fish due to toxicity stress of heavy metals and pesticides have been reported by number of workers (Khan, 1982; Balint *et al.*, 1995; Das *et al.*, 1999; Rao *et al.*, 2000; Khare *et al.*, 2002). In fishes pyrethroid is metabolized and eliminated from the body comparatively more slowly than in mammals or birds (Bradbury *et al.*, 1989).

On the other hand many abiotic factors have been reported to affect the health of the fish. Abiotic factors such as temperature, pH, water column length, turbidity, salinity, alkalinity of water etc. are reported to have profound effect on the reproductive success of the fish (Wilson, 1956; Herzig and Winkler, 1986; Ikuta and Kitamura, 1995; Ernst *et al.*, 2005; Tiffany *et al.*, 2009). Amongst all these abiotic factors, pH is one of the most important factors affecting aquatic life. Levels of pH 4 constitute acidic condition lethal to fish (Ikuta *et al.*, 1992).

Hormones play a critical role in reproduction. Gonadotropin releasing hormone (GnRH) is released from hypothalamus and stimulates the secretion of gonadotropins from the pituitary. Gonadotropins stimulate gonads to secrete steroid hormones leading to the reproductive success. Secretion of hormones such as

gonadotropin releasing hormone (GnRH) in general is lowered by the pesticidal effect, leading to cessation of gametogenesis, vitellogenesis, oocyte maturation, ovulation, spermiation etc. (Lal, 2007).

In the present study we evaluated the combined effects of pH and cypermethrin 25% EC (cypercid 25) on gonads and reproductive hormone (GnRH) of *Puntius javanicus*.

Materials and Methods

The healthy freshwater *Puntius javanicus* (100 ± 5 g; 17-19 cm) were obtained from a local fish reservoir of Barasat, North 24 Parganas, West Bengal, India. The fish were treated with potassium permanganate solution (0.5% w/v) for 1 min to remove any dermal adherent. Fishes were acclimatized for 2 weeks in glass aquarium (500 L) containing de-chlorinated and aerated water at room temperature (32 ± 1.0 C). During acclimatization period commercial food pellet was provided to fishes. Dead fish were removed instantly to keep the water clean. Food was not provided to the fish 24 h prior to toxicity testing and they were starved during the period of experiment. Characteristics of water were measured prior to the experiment with water quality test kit of Aquaponics (Table 1).

Table 1: Water quality parameters

Parameters	Range
Temperature	31-33 C
pH	7.3-7.5
Dissolved oxygen	6.1- 6.7 mg/L (average)
Nitrate	0.18 mg/L (average)
Free carbon dioxide	4.5 mg/L
Alkalinity	118-132 mg/L (as CaCO ₃)
Total hardness	108-116 mg/L (as CaCO ₃)

Toxicity assay:

Water quality was maintained according to the APHA. Six glass aquaria of 40 L capacity were filled with de-chlorinated and aerated water. Ten fish (100 ± 5 g; 17-19 cm) were placed in each of the six aquaria. Cypercid 25 was added at 1, 2, 3, 4, 5 and 6 $\mu\text{g/L}$ concentration in different aquaria. Water of aquaria was changed daily and pesticide concentrations were restored. Fish kept into the pesticide free medium acted as control. The test was performed for 96 h treatment period and dead fish were removed as soon as possible. The number of dead fish per group was recorded against the time of their death as specified by Sprague (1969). Method of Karber as adopted by Dede and Kaglo (2001) was used to calculate the 96 h LC_{50} value of cypercid 25.

Histopathology:

The fish were assigned to six groups, each group having ten specimens (Table 2). pH regulation of water was done by using sodium hydroxide and hydrochloric acid. The water of aquaria was changed daily. Different concentrations of cypercid 25 and different pH were restored daily. After 15 days of exposure to the experimental condition both control and test fishes were taken out of the water and anaesthetized with mild dose of Benzocaine (Larid and Oswald, 1975). The fish were dissected in Ringer's saline to extirpate the gonads. Testes and ovaries were sliced and fixed in Neutral buffered formalin. After dehydration, tissue sections were cut at 4-6 μm and stained with hematoxylin and eosin and photomicrographed by MAGNUS MLX-TR Trinocular microscope and Dewinter Digi Eye Camera.

Determination of GnRH:

Brain tissues were extirpated and rinsed with 1x PBS, homogenized in 1 ml of 1x PBS and stored overnight at -20°C . After 2 freeze-thaw cycles tissue was centrifuged for 5 min at $5000 \times g$ at $2-8^\circ\text{C}$. Supernatant was assayed immediately. ELISA was performed as directed by protocol given by the CUSABIO as per the detection range 0.1 ng/ml- 20 ng/ml with a sensitivity of 0.5 ng/ml. GnRH was quantified by ELISA plate reader (TRANSASIA) at 450 nm wavelength. All determinations were done in triplicate.

Statistical analysis:

Data were presented as mean $\pm$ standard error. Apart from descriptive statistics of the outcome variables, appropriate multivariate statistic such as One-way Anova, LSD was done with Minitab, version 17 Software to understand the causal relationship between parameters.

Results

Toxicity test:

No mortality was recorded in the control group. Fishes were found to be healthy. However, at 1, 2, 3, 4, 5 and 6 $\mu\text{g/L}$ concentrations of cypercid 25, the per cent mortality were 10, 20, 40, 50, 70 and 100 per cent, respectively (Table 3). 96 h LC_{50} of cypercid 25 was calculated and it was found as 3.65 $\mu\text{g/L}$ for *Puntius javanicus* (Table 4).

Testis:

The normal histology of testis of *Puntius javanicus* show seminiferous tubule with distinct, intact, thin fibrous wall. Healthy leydig cells were also seen. Tubules showed scattered tubular cells (Fig. 1a). Testis

Table 2: Experimental groups

10 fishes/ group Exposure period: 15 days	Group	Parameters
	1 (Control)	pH 7 with no Cypermethrin
	2	pH7 + Sublethal concentration Cypermethrin
	3	pH5 + Sublethal concentration Cypermethrin
	4	pH6 + Sublethal concentration Cypermethrin
	5	pH8.5 + Sublethal concentration Cypermethrin
	6	pH9.5 + Sublethal concentration Cypermethrin

exposed to sub-lethal dose of cypercid 25 at pH 7 showed destroyed tubular wall, distorted leydig cell and swollen seminiferous tubule. Intertubular vacuolization and inflammatory cell infiltration was recorded (Fig. 1b). Testis subjected to sub-lethal dose of cypercid 25 at pH 6 and 5 shows much damage of testicular tissues. Swollen seminiferous tubules, inflammation leading to necrosis of the tissue, inter-tubular vacuolization was much prominent. Condensations of tubular cells were seen (Figs. 1c, 1d). Testis exposed to sub-lethal concentration of cypercid 25 at pH 9.5 showed distorted tubular epithelium, condensation of tubular cells, intertubular vacuolation, and distorted leydig cell (Fig. 1e). However, less abnormalities were documented in testis subjected to sub-lethal dose of cypercid 25 at pH 8.5 (Fig. 1f). Vacuolization as well as condensation of tubular cells was not so frequent. Seminiferous tubules were found to be much definite in shape. Small scale infiltration of inflammatory cells was documented.

Ovary:

Normal histology of ovary of *Puntius javanicus*. revealed four stages (I, II, III and IV) of oocytes. Oocyte I had the smallest diameter and oocyte IV had the largest. Few atretic

(AT) cells were found. Intact ovigerous lamellae (OL) and follicular lining (FL) were recorded. Oocyte III cells were found to have provitelline nucleoli (PN) and euvitelline nucleoli (EU) and obvious nuclei (N). Yolk vacuoles (YV) were noticed in Oocyte IV. Among all oocytes oocyte III cells were dominant (Fig. 2a). Ovaries exposed to sub-lethal dose of cypercid 25 at pH 7 shows increased damage of oocytes. Damage includes adhesion (AD) of oocytes, some karyoplasmic clumping (CK), and increased number of atretic (AT) cells. Nuclear retraction (NR) was recorded in Oocyte III. Lifting of the ovigerous lamellae (LOL), Provitelline (PN) and Euvitelline (EU) nucleoli were still found in oocyte III. Size of all types of oocytes were decreased. Patches of macrophage (MI) was seen indicating inflammation (Fig. 2b). Ovary of *Puntius javanicus* subjected to sub-lethal dose of cypercid 25 at pH 6 showed extensive damage of ovigerous lamellae (OL). Numerous atretic cells (AT) were found. Adhesion (AD) could be seen easily. Follicular damage (D), patches of macrophage (MI) and damaged stroma (DS) were noticed. Nuclear retraction (NR) as well as cytoplasmic retraction (CR) was noticed in oocyte III. Oocyte IV was almost absent. An expelled nucleus (EN) was also noticed (Fig.

Table 3: Toxicity testing of Cypercid 25 on *Puntius javanicus* for 96 h

Concentration ($\mu\text{g/L}$)	No. of alive fish				% survival at 96 h	% Mortality at 96 h
	24 h	48 h	72 h	96 h		
0 (Control)	10	10	10	10	100	0
1	10	10	10	9	90	10
2	10	10	9	8	80	20
3	10	10	7	6	60	40
4	9	8	5	5	50	50
5	7	5	5	3	30	70
6	5	2	0	0	0	100

Table 4: Determination of LC_{50} value of Cypercid 25 for *Puntius sp.* for 96 h

Concentration ($\mu\text{g/L}$)	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death X concentration difference
0 (Control)	--	10	0	0	0
1	1	9	1	0.5	0.5
2	1	8	2	1.5	1.5
3	1	6	4	2.5	2.5
4	1	5	5	4.5	4.5
5	1	3	7	6	6
6	1	0	10	8.5	8.5
					$\Sigma = 23.5$

2c). Ovary of treated fish with sub-lethal dose of toxicant at pH 5 exhibited extensive damage of ovigerous lamellae (OL). Large numbers of atretic cells (AT) were found. Adhesion (AD) was profound. Follicular damage (D), patches of macrophage (MI) were seen everywhere. Nuclear retraction (NR) as well as cytoplasmic retraction (CR) was noticed in oocyte III. Oocyte IV was almost absent. An expelled nucleus (EN) was also noticed. Size of all types of oocytes decreased (Fig. 2d). Ovary treated with sub-lethal dose of

toxicant at pH 9.5 showed all types of oocytes, nuclear retraction (NR) was seen in oocyte III. Atretic cells (AT) were recorded in moderate numbers. Lifting of ovigerous lamella (LOL) and macrophage infiltration (MI) was found. Adhesion (AD) was prominent. Size of all oocytes found to be almost the same as control (Fig. 2e). Ovary of *Puntius javanicus* treated with sub-lethal dose of cypercid 25 at pH 8.5 exhibited damage of ovigerous lamellae (OL). Moderate number of atretic cells (AT) was found. Patches of macrophage

Fig. 1a: Testis of control *Puntius javanicus* showing normal histology (H and E X 400), St-Seminiferous tubule, St E-Seminiferous tubule epithelium, Tc- Scattered tubular cells, Lc- Leydig cells. Iv-Intertubular vacuolation

Fig. 1b: Testis of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 7 for 15 days (H and E X 400), d Lc- distorted Leydig cell, Iv- Intertubular vacuolation, St E- seminiferous tubular epithelium

Fig. 1c: Testis of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 6 for 15 days (H and E X 400), St E- Seminiferous tubular epithelium, In- Inflammation, Ctc- Condensation of tubular cells, D Lc- Distorted leydig cell.

Fig. 1d: Testis of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 5 for 15 days (H and E X 400), Dse- Inflammation and distortion of seminiferous epithelium, Ctc- Condensation of tubular cells, Iv- Intertubular vacuolation

Fig. 1e: Testis of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% at pH 9.5 for 15 days (H and E X 400), d St E- distorted tubular epithelium, Ctc- Condensation of tubular cells, Iv- Intertubular vacuolation, D Lc- Distorted leydig cell.

Fig. 1f: Testis of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 8.5 for 15 days (H and E X 400), St- Seminiferous tubule, In- Inflammation, Iv- Inter tubular vacuolation.

Fig. 2a: Ovary of control *Puntius javanicus* showing normal histology (H and E X 100). O I- oocyte I, O II- oocyte II, O III- oocyte III, O IV- oocyte IV, N- nucleus, PN- Provitelline and EU- euvitelline nucleoli, FL- follicular linings YV- yolk vacuoles . AT - Atretic cells.

Fig. 2b: Ovary of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 7 for 15 days (H and E X 100). LOL-lifted ovigerous lamellae, NR- Nuclear retraction, AT- Atretic cell, MI- Macrophage infiltration, D-Damaged oocyte

Fig. 2c: Ovary of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 6 for 15 days (H and E X 100).OL- Ovigerous lamellae, AT- Atretic cells, AD- Adhesion, D- Follicular damage, MI- Macrophage infiltration, DS- Damaged stroma, NR- Nuclear retraction, CR- Cytoplasmic retraction, EN- Expelled nucleus

Fig. 2d: Ovary of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 5 for 15 days (H and E X 100). OL,- Ovigerous lamellae, AT- Atretic cells, AD- Adhesion , D- Follicular damage, MI- Macrophage infiltration, NR- Nuclear retraction, CR- Cytoplasmic retraction, EN- Expelled nucleus.

Fig. 2e: Ovary of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 9.5 for 15 days (H and E X 100). NR- Nuclear retraction, AT- Atretic cells, LOL- Lifting of ovigerous lamellae, MI- Macrophage, AD- Adhesion.

Fig. 2f: Ovary of *Puntius javanicus* treated with sub-lethal dose of Cypermethrin 25% EC at pH 8.5 for 15 days (H and E X 100). LOL- Lifting ovigerous lamellae, AT- Atretic cells, MI- Macrophage infiltration, NR- Nuclear retraction in oocyte III, CK- Karyoplasmic clumping.

(MI) were seen. Nuclear retraction (NR) was noticed in oocyte III. Karyoplasmic clumping (CK) was seen in oocyte IV. Size of all oocytes found to be same as control (Fig. 2f).

GnRH concentration:

Fish exposed to sublethal concentration of cypercid 25 at different pH exhibited significant ($p < 0.001$) variation of concentration of GnRH (Table 5). GnRH level in acidic water (pH 6 and pH 5) with toxicant showed much lower concentration (0.400 ± 0.012 $\mu\text{g/ml}$ and 0.517 ± 0.018 $\mu\text{g/ml}$, respectively) in comparison to the fish exposed to the basic water (pH 8.5 and pH 9.5) with toxicant (1.020 ± 0.057 $\mu\text{g/ml}$ and 0.743 ± 0.018 $\mu\text{g/ml}$, respectively) (Table 6). Significant ($p < 0.05$) decrease of GnRH was recorded in fish exposed to cypercid 25 and gradual decrease was noticed in acidic (pH 5 and pH 6) and alkaline medium (pH 9.5). However, in pH 8.5, concentration of GnRH increased significantly ($p < 0.05$) (Fig. 3). The GnRH concentration at pH 8.5 with toxicant (1.020 ± 0.057 $\mu\text{g/ml}$) was found to be near the concentration of GnRH of fish exposed to pH 7 without toxicant (1.150 ± 0.029 $\mu\text{g/ml}$) (Table 7).

Discussion

In the present study 96 h LC_{50} values of cypercid 25 for *Puntius javanicus* was recorded as 3.65 $\mu\text{g/L}$. For various pyrethroids the range of 96 hr LC_{50} value has been reported to be below 10 $\mu\text{g/L}$ (Smith and Stratton, 1986; Bradbury and Coats, 1989). Several investigators have reported 96 h LC_{50} values of cypermethrin in different fish species: 7.2 $\mu\text{g/L}$ for *Heteropneustes fossilis* (Mishra *et al.*, 2002); 0.9–1.1, 1.2, 0.5, 0.4 and 2.2 $\mu\text{g/L}$ for *Cyprinus carpio*, *Salmo trutta*, *Salmo gairdneri*, *Scardinius erythrophthalmus*,

and *Tilapia nilotica*, respectively (Bradbury and Coats, 1989).

Reproductive function analysis is very important to determine the dynamics of fish populations (Mekkawy and Hassan, 2011; Ojaveer *et al.*, 2015). Exposure of pollutants in aquatic medium adversely affect fish reproduction leading to reproductive impairment (Aquezar *et al.*, 2006; Ebrahimi and Taherianfrad, 2011; Gerbron *et al.*, 2014; Zhang *et al.*, 2016). Testis of fish exposed to sub-lethal dose of cypercid 25 at pH 7 shows destroyed tubular wall, distorted leydig cell and swollen seminiferous tubules. Inter-tubular vacuolization and inflammatory cell infiltration are suggestive of initiation of necrosis. When fish were subjected to the acidic medium (pH 5 and pH 6) and in extreme basic medium (pH 9.5), above mentioned damages were found to be more profound. However, in pH 8.5 fish were found to retain normal architecture of testis with few abnormalities. Gross inflammation was found in testicular tissue in the present study. In aquatic animals subjected to environmental toxicants testicular inflammation was found to be most obvious response (Sokal *et al.*, 1985; Zutshi and Murthy, 2001). Detrimental effect of cypermethrin on the gonadal morphology and histology was reported by several workers. A few of them are -- decreased spermatozoa and abnormal testicular connective tissue in Japanese medaka *Oryzias latipes* (Yokota *et al.*, 2000), loss of testicular structure in Japanese medaka *Oryzias latipes* (Metcalfe *et al.*, 2001), gonad histology alteration in adult common carp *Cyprinus carpio* (Mardich *et al.*, 2007). In *Oreochromis mossambicus* BHC exposure caused hyperplastic changes in both primary and secondary spermatocytes (Pandey and Shukla, 1980). Likewise hyperplasia and vacuolization

Table 5: One-way Anova for GnRH concentration in experimental fish, *Puntius javanicus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.238311111	5	0.247662	101.0866	0.0000000
Within Groups	0.0294	12	0.00245		
Total	1.267711111	17			

Table 6: Concentration of GnRH in different experimental groups of *Puntius javanicus*

Experimental groups	GnRH concentration ($\mu\text{g/ml}$)
pH 7, No toxicant (Control 1)	1.150 $\pm$ 0.029
pH 7, Toxicant (Control 2)	0.837 $\pm$ 0.009
pH 5, Toxicant	0.400 $\pm$ 0.012
pH 6, Toxicant	0.517 $\pm$ 0.018
pH 8.5, Toxicant	1.020 $\pm$ 0.057
pH 9.5, Toxicant	0.743 $\pm$ 0.018

Table 7: Post hoc Test (LSD) Multiple Comparison

(I) elisa_res	(J) elisa_res	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
					Lower Bound
pH 7, No toxicant (Control 1)	pH 7, Toxicant (Control 2)	0.313333	0.040415	0.000005	0.225278
	pH 5, Toxicant	0.750000	0.040415	0.000000	0.661944
	pH6, Toxicant	0.623400	0.040415	0.000000	0.545278
	pH 8.5, Toxicant	0.130000	0.040415	0.007400	0.041944
	pH 9.5, Toxicant	0.406667	0.040415	0.000000	0.318611

Fig. 3: Concentration of GnRH in different experimental groups of *Puntius javanicus*

in all types of spermatogenic cells have been reported in *Sarotherodon mossambicus* exposed to dimecron (Lakhani and Pandey, 1985). Endosulfan also causes testicular degeneration and disappearance of sperm (Singh and Sahai, 1987). These reports are in good agreement with the findings of present study.

Ovaries of fish exposed to sub-lethal dose of cypercid 25 at pH 7 showed increased amount of damage such as adhesion (AD) of oocytes, karyoplasmic clumping (CK), and a higher percentage of atretic (AT) cells. Nuclear retraction (NR) in oocyte III is the indication of damage in oocyte III. Sizes of all types of oocytes were decreased. Patches of macrophage (MI) is seen indicating inflammation. When fish are subjected to the acidic medium (pH 5 and pH 6) and in extreme basic medium (pH 9.5) damages were found to be more profound. However, in pH 8.5 fish were found to retain normal architecture of ovaries with few abnormalities. Similar results were reported

by other workers. Retardation of ovarian activity and decrease in diameter of oocyte cells due to *in vivo* exposure of endosulfan was reported by Pandey (1988). Damage is directly correlated with atresia which in turn correlated with a reduced fecundity (Agarwal and Singh, 1990). Follicular atresia found in present study is also reported by other workers (Molina *et al.*, 2013; Senarat *et al.*, 2017). The reduction of oocyte diameter due to stressor found in present study is also supported by findings of other workers (Alquezar *et al.*, 2006; Teta and Naik, 2017). Necrosis found in the present study makes the formation of viable eggs difficult (Dutta and Maxwell, 2003). Partial disruption of the ovarian follicles, vacuolation in the cytoplasm of germinal cells, and secondary oocytes were reported in *Heteropneustes fossilis* exposed to BHC (Hazarika and Das, 1998). Degeneration of follicular walls, connective tissues and vacuolization in the cytoplasm of stage II and stage III oocyte was observed in *Anabas testudineus* treated with carbofuran

(Chatterjee *et al.*, 1997). This supports the present study that the oocytes were affected with a degeneration of connective tissue, disruption of follicles, and vacuolization in the cytoplasm.

One-way Anova showed significant ($p < 0.001$) difference in the concentration of GnRH in different experimental groups. GnRH level decreased significantly ($p < 0.05$) in fishes exposed to cypercid 25 compared to control. These findings are with good agreement with findings of several researchers (Kapur and Toor, 1978; Singh and Singh, 1987; Skandhan *et al.*, 2001; Maxwell and Dutta, 2005). GnRH level in acidic water (pH 5 and pH 6) with toxicant showed much lower concentration in comparison to the fishes exposed to the basic water (pH 8.5 and pH 9.5) with toxicant. The GnRH concentration at pH 8.5 with toxicant found to be almost equal to the concentration of GnRH of fishes exposed to pH 7 without toxicant.

Conclusion

Present study documented alteration of histoarchitecture of testis and ovary and concentration of GnRH due to exposure of sub-lethal dose of cypermethrin 25% EC. These alterations were very much influenced by alteration of pH of water. Fish exposed to the basic water (pH 8.5) found to be somehow able to minimize the alteration induced by the toxicant in comparison with the fish exposed to acidic water (pH 5 and 6) and extreme basic water (pH 9.5). Fish maintained in pH 8.5 shows much less alterations regarding the histoarchitecture of gonads as well as concentration of GnRH.

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